REMARKS

Claims 9, 10, 12 and 17 have been amended. Claim 11 has been canceled. Claims 1-10, 12-14 and 16-24 currently are pending. The examiner withdrew claims 1-8, 13-14, 16 and 19-24 from consideration.

Restriction requirement

The examiner maintained the restriction requirement because the examiner believes it is well-established in the art that modification of at least one amino acid imparts modified substrate specificity since the structure of a protein denotes its function. The examiner therefore believes the substrate specificity of the various mutants of Munro et al. are different from the specificity of the wildtype.

In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic **necessarily** flows from the teaching of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990). The examiner has not done so in the present case. Therefore, applicants believe the current restriction requirement is improper.

35 USC § 112, second paragraph

Claims 11-12 and 17-18 are rejected under 35 USC § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants correct a typing error in claim 10. It now reads "N- or S heterocyclic...

compounds."

Applicants insert a conjuction in claim 12 .

The examiner stated claims 17-18 recite the limitation "at least one compound selected from the groups a) to d) of compounds defined above" but there is insufficient basis for this limitation in the claim. In response, applicants insert into claim 17 a definition of the compounds selected from group a) in accordance with the original wording of claim 1. Reference to compounds of groups b), c) and d) has been deleted from claim 17.

The examiner believes claims 9-12 and 17-18 omit the essential steps of converting non-heterocyclic aromatic substrates into heterocyclic aromatic compounds.

Applicants point out that after going through steps a1) and a2) the oxidation product can be isolated because the conversion has occurred.

35 USC § 102

Claims 9-10 and 17-18 are rejected under 35 USC § 102(b) as being anticipated by Wong et al. The examiner believes this reference teaches a method of oxidizing – or S- heterocyclic mono- or polynuclear aromatic compounds with a $P450_{CAM}$, cytochrome P450 monooxygenase from *Pseudomonas putida* (pages 2-3, 7-9 and 13-31) and the method of Wong et al. is carried out at a temperature of about 20-40°C, a pH of about 6-9 and a reaction medium containing about 10-100 molar excess of the substrate.

Applicants amend claim 9 by introducing the subject matter of claim 11 into claim

9. Applicants delete claim 11.

Anticipation can only be established by a single prior art reference which discloses each and every element of the claimed invention. *RCA Corp. v. Applied Digital Data Systems, Inc.*, 730 F.2d 1440, 1444, 221 USPQ 385, 388 (Fed. Cir. 1984). As the amended, the present claims are therefore not anticipated by Wong et al. The claimed process for the microbiological oxidation of an N - or S- heterocyclic mono- or polynuclear aromatic compound by means of cytochrome P450 monooxygenase BM-3 mutants is anticipated by Wong et al.

35 USC § 103

Claims 9 and 11-12 were rejected under 35 USC § 103(a) as being unpatentable over Wong et al. in view of Graham-Lorence et al. The examiner states that the difference between the reference of Wong et al. and the present invention is that Wong et al. do not teach a method of oxidizing heterocyclic aromatic compounds using mutant P450 BM-3, cytochrome P450 monooxygenases from *Bascillus megaterium* but that it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to use the mutant P450 BM-3 of Graham-Lorence et al. to oxidize heterocyclic aromatic compounds.

Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. MPEP 2143.01.

Applicants believe the examiner has not properly combined the cited references. Specifically, applicants believe the examiner engages in hindsight reasoning. There must be a reason apparent at the time the invention was made to a person of ordinary skill in the art for applying the teaching at hand, or the use of the teaching as evidence of obviousness will entail prohibited hindsight. *In re Nomiya, Kohisa, and Matsumura*, 509 F.2d 566, 184 USPQ 607 (CCPA 1975).

The cytochrome P450 monooxygenases are divided up, depending on their electron transfer system, into four difference classes enzymes. Cytochrome P450 monooxygenase BM-3 forming the basis of the present invention as well as referred to by Graham-Lorence, belongs to P450 monooxygenases of class II while $P450_{CAM}$ referred to by Wong et al. belongs to a different class of enzymes, namely class I. Moreover, said two enzymes are completely different with respect to their natural substrate. While the P450 monooxygenase BM-3 of the present invention usually catalyzes the subterminal hydroxylation of long-chain, saturated acids (see paragraph 4, page 1 of the present specification), $P450_{CAM}$ is considered to be highly substrate specific and catalyzes the regio- and stereo- selective hydroxylation of camphor to 5-exo-hydroxycamphor (see Wong et al., page 1, last paragraph). In view of this, applicants do not see on what basis the examiner concludes that these two enzymes may be regarded as being "homologous" (page 5, last paragraph of the office action).

In line with said different substrate specificities of natural $P450_{CAM}$ and P450 BM-3, the crystal structure of $P450_{CAM}$ reveals a compact substrate binding pocket

while the binding pocket of P450 BM-3 consists of a long, hydrophobic channel extending from the distal face of the heme to the protein surface. Therefore, the substitutions at position 87 of $P450_{CAM}$ cannot have the same effect as the nominally similar substitution in position 87 of P450 BM-3.

Also, position Phe87 of P450 BM-3 is highly conserved. It is located in the active site of the protein and is of importance for the correct orientation of the fatty acid hydrocarbon chain. Comparison of substrate-free and substrate-bound crystal structures of PM450 BM-3 revealed a substantial conformational difference that is caused by the phenyl ring of phenyl alanine residue. Mutations of P450 BM-3 at position 87 therefore may affect its activity and stereo- or regio- selectivity as confirmed by the results of Graham-Lorence. It was observed by Graham-Lorence et al. (see page 1127, left column, 3rd paragraph) that "...replacement of phynylalanine 87 with valine converted cytochrome P450 BM-3 into a regio- and stereo- selective arachidonic acid epoxygenase..."

This is the key result of Graham-Lorence et al. with respect to mutation in position 87. Arachnidonic acid structurally is completely different from N - or S - heterocyclic compounds as used according to the present invention. Moreover, this reference does not provide any evidence which would allow a skilled reader to reasonably expect that substrate specificity of said class II monooxygenase may be altered by the same mutation so that oxidation of N - or S - heterocyclic mono-or polynuclear aromatic compounds will become possible, as surprisingly observed in the

present invention.

For the reasons expressed above, it is urged that the prior art references cited by the examiner either singly or in combination fail to anticipate or suggest the present invention as defined by the amended claims. Accordingly, a *prima facie* case of obviousness has not been established by the examiner, and the rejection under 35 USC § 103 should be withdrawn.

A check in the amount of $420.00 is attached to cover the required two month extension fee.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such deposit account.

Respectfully submitted,

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COMPLETE LISTING OF CLAIMS IN APPLICATION

1-8. (withdrawn)

9. (currently amended) A process for the microbiological oxidation of N - or S - heterocyclic mono- or polynuclear aromatic compound which comprises

a1) culturing a recombinant microorganism which expresses a cytochrome P450 monooxygenase of bacterial origin in a culture medium, in the presence of an exogenous or intermediately formed substrate; or

a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase of bacterial origin; and

b) isolating the oxidation product formed or a secondary product thereof from the medium

, and wherein the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO: 2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43,48-52, 67-70, 330-335, 352-356, 73-82 and 86-88.

10. (currently amended) A process as claimed in claim 9, wherein the exogenous or intermediately formed substrate is selected from optionally substituted- N - or S- heterocyclic mono- or polynuclear aromatic compounds.

11. (canceled)

12. (currently amended) A process as claimed in claim 9 ~~11~~, where the mutant has at

least one of the following mono- or polyamino acid substitutions:

a) Phe87Val;

b) Phe87Val, Leu 188Gln~~; or~~ , and

c) Phe87Val, Leu188Gln, Ala74Gly.

13-14. (withdrawn)

15. (canceled)

16. (withdrawn)

17. (currently amended) A process as claimed in claim 9, wherein, as exogenous substrate, at least one compound selected from the group~~s~~ a) ~~to d)~~ of compounds defined ~~above~~ claim 1 is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.

18. (original) A process as claimed in claim 17, wherein, as exogenous substrate, a compound selected from indole, n-hexane, n-octane, n-decane, n-dodecane, cumene, 1-methylindole, α-, β-, or γ-ionone, acridine, naphthalene, 6-methyl- or 8-methylquinoline, quinoline and quinaldine is employed.

19-24. (withdrawn)